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Analogs of α-conotoxin PnIC are selective for α7β2 over α7-only subtype nicotinic acetylcholine receptors

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Objective/Aim/Hypothesis: The objective of this work is to discover and characterize the first ligands able to identify selectively nicotinic acetylcholine receptors assembled from α7 and β2 subunits (α7β2-nAChR). Basal forebrain cholinergic neurons (BFCNs) express α7β2-nAChR. Elevated concentrations of oligomeric amyloid-β (associated with early Alzheimer's disease) appear to mediate BFCN neuronal dysfunction. Expression of α7β2-nAChR by additional important cholinergic and GABAergic neuronal circuits of the central nervous system has been observed. However, further studies are stymied by a lack of ligands that can positively identify α7β2-nAChR from the related and more widespread α7-only homomeric nAChR subtype. This problem arises since α7-only- and α7β2-nAChR share identical agonist binding sites, located at α7/ α7 subunit interfaces, that are the targets of most current ligands. We hypothesized that new α-conotoxin (α-Ctx) ligands may instead selectively inhibit α7β2-nAChR via α7/β2 subunit interfaces.

Design/Approach/Methods: Two-electrode voltage clamp (TEVC) electrophysiology was used to screen ≫500 novel α-Ctxs for selectivity towards α7β2- over α7-only-nAChR functionally expressed in *Xenopus* oocytes. Kinetics analysis was used to probe the subtype selectivity and mechanism of α-Ctx antagonism. Molecular dynamics (MD) simulations were used to identify amino-acid residues at putative α7/β2 subunit α-Ctx binding sites. Site-directed mutagenesis was used to probe these hypothesized sites. TEVC electrophysiology was also used to determine α-Ctx activity at non- α7 nAChR subtypes.

Results: We discovered α-CtxPnIC analogs that selectively antagonize α7β2- over α7-only-nAChR. Kinetics analysis showed that association rates were similar across α-CtxPnIC analogs, and between α7β2- and α7-only-nAChR subtypes. Slower disassociation from α7β2- *vs*. α7-only-nAChR mainly drove selectivity towards α7β2-nAChR. The α-CtxPnIC [S4R] and [L10Y] analogs were the most selective towards α7β2-nAChR (18- and 57-fold vs. α7-only-nAChR, respectively). MD identified two sets of β2 subunit residues at the putative α7/β2 subunit interface for α-CtxPnIC analogs that differed from those at the known α7/ α7 subunit interface competitive agonist site. Mutating either set of β2 subunit residues to their α7 subunit equivalents partially reduced α-CtxPnIC [S4R] selectivity towards α7β2-nAChR. Activity of α-CtxPnIC analogs was generally low at non- α7-nAChR subtypes; data across analogs suggested approaches to decrease off-target affinity further.

Conclusions: We have identified the first ligands with selectivity towards α7β2-nAChR and proved a prototypical example of non-competitive antagonism by α-Ctxs. This discovery profoundly expands the scope of application of α-Ctx ligands (which have already provided important nAChR research and translational breakthroughs). Further development of α-CtxPnIC analogs will enhance α7β2-nAChR selectivity, providing opportunities for basic and translational scientific breakthroughs related to nAChR biology, Alzheimer's disease, and cholinergic contributions to cognition.

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